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(56) Documents Cited
EP 0293892 A2 EP 0184162 A2 US 5164495 A

(58) Field of Search
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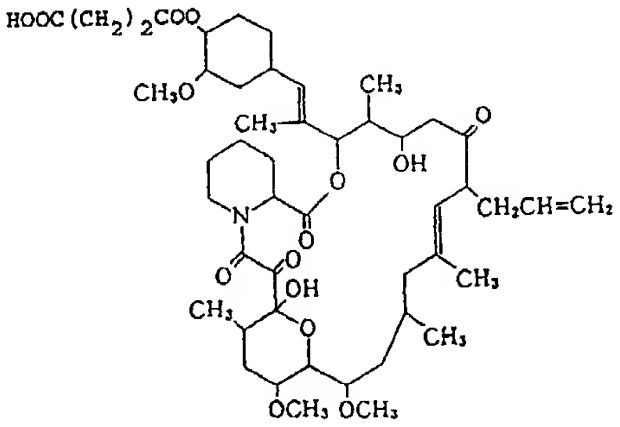
(54) Process for producing half esters of the macrolide FK506

(57) Half esters of the compound FK506 with dicarboxylic acids are produced by reacting a mixture comprising FK506 and a dicarboxylic acid or anhydride thereof, such as succinic anhydride, in the presence of a dialkylaminopyridine such as 4-dimethylaminopyridine, in the absence of other bases.

The FK506 half esters thus produced are useful as intermediates in the preparation of various conjugates for utilization in diagnostic assays for the compound FK506. The succinate ester has the formula:-



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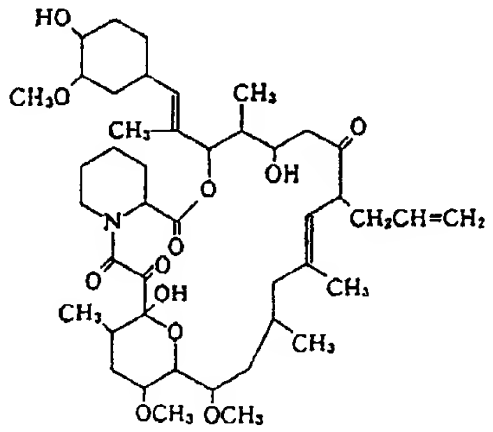
NEW PROCESS

This invention relates to an improved preparation process for producing a starting materials which can be utilized in the preparation of components for diagnostic assays for determining the presence and/or amount of FK506 in biological fluid such as from patient samples. More particularly, the present invention relates to a preparation process for producing reaction products of FK506 with dicarboxylic acids or anhydrides thereof.

It is well known that FK506 of the following formula:



, which is produced by fermentation of Streptomyces tsukubaensis No. 9993 (which has been deposited at Fermentation Research Institute, Agency of Industrial Science and Technology under the deposit No. FERM BP-927), is useful for treatment of rejection by transplantation, autoimmune diseases, etc. (EP 0 184 162-A2). And, techniques for monitoring the blood concentration of FK506 has also been studied in order to control the blood concentration of FK506 most effectively (EP 0 293 892-A2).

During those studies, it was understood that half esters of FK506 with a dicarboxylic acid or anhydride thereof (hereinafter referred as FK506 half-esters) were very important. Those FK506 half-esters are particularly useful as intermediates in the preparation of various conjugates for utilization in diagnostic assays for FK506. For example, the FK506 half-esters can be converted into a covalent conjugates of FK506 with enzyme such as alkaline phosphatase, and further the FK506 half-esters can also be usable in preparation of various conjugates with various poly(amino)acids such as bovine serum albumin (BSA), which are useful as immunogens for raising antibodies (EP 0 293 892-A2 and USP 5 164 495).

Accordingly, the production of high yields of FK506 half-esters is economically advantageous when its amounts necessary for commercial scale production are contemplated. For example, the above USP 5 164 495 tells that the yield of the FK506 half-esters could be improved by using triethylamine compared with the process using pyridine which is described in the prior EP 0 293 892-A2 .

As a result of an extensive study, the inventor of the present invention could found that FK506 half-esters could be produced in a quite high yield, even if without using the above triethylamine or pyridine. So, the present

inventor has succeeded in providing an further improved preparation process for producing FK506 half-ester which will reliably yields improved amounts of FK506 half-esters, and at the same time, reducing the amount of undesirable side products.

Accordingly, the present invention provides a new preparation process for production of half esters of FK506, i.e., FK506 half-esters, with dicarboxylic acids or anhydrides thereof, by reacting mixture comprising FK506 and dicarboxylic acids or anhydrides thereof in the presence of dialkylaminopyridine without any other bases such as pyridine or triethylamine.

Dicarboxylic acids and anhydrides usable in the present invention are of relatively low molecular weight, i.e., having a molecular weight in the range of from 90 to 250, preferably in the range of from 100 to 200. Examples of dicarboxylic acids include oxalic acid, adipic acid, glutaric acid, maleic acid, maleic anhydride, fumaric acid, succinic acid, succinic anhydride, terephthalic acid, terephthalic anhydride, hexahydroterephthalic acid and hexahydrophthalic anhydride. The most preferred one is succinic anhydride.

The reaction of the dicarboxylic acid or their anhydride with FK506 is carried out in a temperature range of from 5 to 30 degrees Celsius, preferably from 20 to 25 degrees Celsius, for a period of about a few hours. Preferably the reaction is carried out at atmospheric temperature and pressure.

The reaction is usually conducted in a conventional solvent which does not adversely influence the reaction such as methylene chloride. And the object products can be isolated and purified in a conventional manner.

The suitable alkyl group in "dialkylaminopyridine"

may include C_1 to C_6 alkyl group such as methyl, ethyl, etc. The most preferred "dialkylaminopyridine" is 4-dimethylaminopyridine.

The following example is given for the purpose of illustrating the present invention.

Example

Preparation of a half ester of FK506 with succinic anhydride

FK506 (0.2 g; 0.24 mmole), succinic anhydride (28 mg; 0.28 mmole) and 4-dimethylaminopyridine (92 mg; 0.74 mmole) were dissolved in dry methylene chloride (3 ml) and stirred at room temperature for 1 hr, followed by another addition of succinic anhydride (21 mg, 0.21 mmole). The reaction mixture was further stirred at room temperature for 2 hrs. The reaction was followed by SiO_2 TLC as described in Fujisawa's EP 0 293 892-A2. The formation of by-products was significantly suppressed. The reaction mixture was extracted into 20 ml of ethyl acetate with the addition of brine (10 ml). The aqueous phase was further extracted with ethyl acetate (10 ml x 2). The ethyl acetate layer was combined, dried over $MgSO_4$ and concentrated under reduced pressure to give an oily residue which was subjected to SiO_2 column chromatography purification as described in EP 0 293 892-A2. The half-ester of FK506 with succinic anhydride, i.e. FK506-hemisuccinate, was obtained quantitatively. The obtained product was confirmed as the same compound as the FK506-hemisuccinate shown in EP 0 293 892-A2 by the conventional manner.

CLAIMS

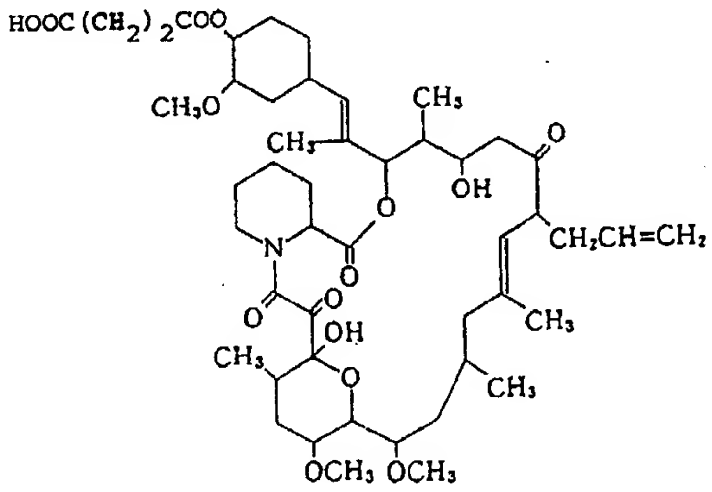
What we claim is :

1. A process for production of half-ester of FK506 with a dicarboxylic acid or anhydride thereof by reacting a mixture comprising FK506 and a dicarboxylic acid or anhydride thereof in the presence of dialkylaminopyridine without any other bases.

2. The process of claim 1, in which the dialkylaminopyridine is dimethylaminopyridine.

3. The process of claim 1 or 2, in which the dicarboxylic acid or anhydride thereof is succinic anhydride.

4. The process of claim 3, in which the half-ester of FK506 is the compound having the following formula.



Patents Act 1977
Examiner's report to the Comptroller under Section 17 -6-
(The Search report)

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Search Examiner
D S LUCAS

Date of completion of Search
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Databases (see below)
(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ONLINE DATABASE : CAS ONLINE

Documents considered relevant following a search in respect of Claims :-
1-4

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
X	EP 0293892 A2	(FUJISAWA) - see particularly Example 1	1-4
X	EP 0184162 A2	(FUJISAWA) - see particularly Example 12	1-4
X	US 5164495 A	(ABBOTT) - see particularly Claim 1 and Example 3	1-4

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).